Incorporation-by-reference of materials submitted on a compact disk.

Applicants have submitted Sequence Listing on machine readable media with the filing of the application.

Background of the Invention

B1

(1) Field of the Invention

The present invention relates to compounds, some of which may be indirectly cytotoxic combinations of compounds, which have a high avidity for, and can be targeted to, selected cells.

~~Background~~ (2) Description of Related Art including Information Disclosed under 37 CFR 1.97 and 1.98

On page 10, after the first full paragraph and before the second full paragraph, please insert:

B2

Brief Description of the Drawings

Page 38, starting at line 25

Figure 1 shows schematic examples of ~~the~~ various types of compounds of the invention.

Figure 2 shows a schematic diagram ~~showing~~ of the construction of the gene of the invention~~expressing the HMFG-1 Fab-Caspase fusion protein. Steps A to G are described in the text below. Note that the final construct pET20HMFGLSCasp3 contains the VL-CL expression cassette in the Bpu1102 I site. An alternative construct, lacking the pel B secretion signal for cytosolic expression is constructed the same way, using pET21b as the starting vector, and the final clone is called pET21HMFGLSCasp3.~~

B3

Figure 3 shows a ~~the~~ continuous nucleotide sequence ~~of the construct which when expressed in *E. coli*, will assemble to form a HMFG1 Fab/Caspase-3 fusion protein. The vector backbone is pET20b. Sequence reads as follows:~~

~~Pel B secretion signal (underlined) 1-66; VH domain (italics) 67-420; CH1 domain 421-714; Linker peptide (underlined) 715-759; re-arranged caspase-3/CPP-32 (italics) 760-~~

~~1277; Linker and Polyhistidine Tag sequence (underlined) 1278-1320. Additional T7 promoter, and ribosome binding site 1321-1461; Pel B secretion signal (underlined) 1462-1527; VL domain 1462-1869; CL domain 1870-2193.~~

~~The restriction sites referred to in Figure 2 are presented in bold in Figure 3~~.

Figure 4 shows a schematic diagram showing the construction of a ~~the~~ vector ~~(pET20scFvCasp3) expressing the scFv rearranged Caspase 3 fusion protein. Steps A to E are described in the text. An alternative construct, lacking the *pel* B secretion signal for cytosolic expression is constructed the same way, using pET21b as the starting vector, and the final clone is called pET21scFvSCasp3. Flanking amino acid sequences are shown~~.

B3

Figure 5 shows a ~~the~~ continuous nucleotide and amino acid sequence of a ~~the~~ fusion gene ~~which when expressed in *E. coli*, will produce the scFv- rearranged caspase 3 fusion protein. The vector backbone is pET20b. Sequence reads as follows: end of *pel* B from pET20b vector (1-8), scFv (9-731), linker (732-752), rearranged caspase (753-1652), His-tag (1653-1673)~~.

Figure 6 shows an expression in *E. coli* ~~BL21(DE3)pLysS~~ of the ~~scFv- rearranged~~ caspase 3 fusion protein ~~from vector pET20scFvcasp3. P=cell pellet, SN=bacterial supernatant, M=molecular weight markers. Time course is 0 to 16 hrs. Arrow indicates position of scFv-rearranged caspase 3 protein~~.

Figure 7 shows ELISA on pure CEA ~~of the scFv and scFv- rearranged caspase 3 fusion protein, developed with anti-HIS antibody (Qiagen). The starting concentration of the proteins were 1 μg/ml in PBS~~.

Figure 8 shows a PARP cleavage assay ~~of the scFv and scFv- rearranged caspase 3 fusion protein a cell lysate from SKOV3 cells, which contains full length PARP is incubated with the tested proteins for 1 hr at 37 °C. The full length (arrow A, 130 KDa) and cleaved PARP (arrow B, 85 KDa) are detected by western blot. Lanes are as follows: (1) Cell lysate, untreated, (2) Cell lysate treated with scFv-rearranged caspase fusion protein, (3) Cell lysate treated with scFv only, (4) Cell lysate treated with pure caspase. Detecting antibody as anti-PARP (Santa Cruz biochemicals Ltd)~~.

Figure 9 shows cytotoxicity of 7 selected ~~scFv~~-caspase 3 fusion proteins ~~and the anti-CEA scFv alone. Fifty and ten microlitres of concentrated bacterial supernatant were added to LS174T for 48 hr. Background values for untreated cells were subtracted to give actual cell lysis values as measured by the Cytotox 96 kit~~.

B3

Detailed Description of the invention